Reflections on flurbiprofen eyedrops

van Sorge, Adriaan Alastair

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Adriaan Alastair van Sorge

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PROMOTORES	Prof. dr. J. Zaagsma Prof. dr. W.J. Quax Prof. dr. H.W. Frijlink
CO-PROMOTOR	Dr. N.J. van Haeringen
BEOORDELINGSCOMMISSIE	Prof. dr. P.T.V.M. de Jong Prof. dr. J.R.B.J. Brouwers Prof. dr. H.V. Wikström

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My parents, who got me started

Aty, Nina, Joline and Arlette, who let me go on

Eelco, who wouldn't let me quit

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REFERENCES

1. van Sorge AA, Wijnen PH, van Delft JL, Carballosa Coré-Bodelier VMW, van Haeringen NJ. Flurbiprofen, S(+), eyedrops: formulation, enantiomeric assay, shelflife and pharmacology. Derived from Pharm World Sci 1999;21:91-5.
2. Sorge van AA, Delft van JL, Bodelier VMW, Wijnen PH, Haeringen van NJ. Specificity of flurbiprofen and enantiomers for inhibition of prostaglandin synthesis in bovine iris/ciliary body. Prostaglandins Other Lipid Mediat 1998;55:169-77.
3. Haeringen van NJ, Sorge van AA, Delft van JL, Carballosa Coré-Bodelier VMW. Flurbiprofen and enantiomers in ophthalmic solution tested as inhibitors of prostanoid synthesis in human blood. J Ocular Pharmacol 2000;16:345-52.
4. Haeringen van NJ, Sorge van AA, Carballosa Coré-Bodelier VMW. Constitutive cyclooxygenase-1 and induced cyclooxygenase-2 in isolated human iris inhibited by S(+) flurbiprofen. J Ocular Pharmacol 2000;16:353-61.
5. Sorge van AA, Etten van RJ, Rehmann CJ, Rijnders AJM, Haeringen van NJ. ^{99m}Tc-Diflunisal and the human iris: topical application reveals localization. J Ocular Pharmacol 2002;18:185-95.
6. Sorge van AA, Ruiken IWM, Janssen HWM, Haeringen NJ. S(+) flurbiprofen and R(-) flurbiprofen. ^{99m}Tc-labeling reveals difference in stereochemistry. Enantiomer 2002; Accepted pending suitable revision.
7. Dröge MJ, van Sorge AA, van Haeringen NJ, Quax WJ, Zaagsma J. Alternative splicing of cyclooxygenase-1 mRNA in the human iris. Submitted.

PREFACE

A HISTORICAL INTRODUCTION

A simple question put forward in 1980 by one of the ophthalmologists to the hospital pharmacist led to a chain of events culminating in this thesis. The question was: "Is it possible to prepare indomethacin eyedrops?".

The principal reason for the question were reports on eye research, mainly of Japanese origin (1, 2, 3, 4, 5), indicating that use of topically applied indomethacin could prevent cystoid macular edema after lens extraction, required e.g. when a patient had acquired a senile cataract. The incidence of this complication varied between 2 and 50% but reports of 70% were known as well. The complication had been reported earlier as a newly defined vitreous syndrome following cataract surgery and was described in 1953 (6).

Just over 65 years ago it was postulated by Selye (7) that our physiological system, activated by stress, not only will try to protect and restore itself but also can derail and afflict damage. The most common responses to stress are activation of the sympathetic nervous system and of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in or accompanied by immunological changes.

The immunological defense mechanisms of the ocular surface have been reviewed in detail in 1983 (8). A review ten years earlier (9) refers to the finding by Ambache of a physiological smooth muscle stimulant as a constituent of the rabbit iris ("Irin") in 1957, and the further elucidation of its nature in 1959 (10,11).

In 1967 the synthesis of prostaglandins in the pig iris was reported (12) and in 1968 their release from bovine iris (13). Subsequently, prostaglandins were related to various ocular functions indeed (14).

In 1971 a pivotal study was reported by Vane (15) demonstrating the inhibiting effect on prostaglandin synthesis as the mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs).

Release of prostaglandins in the rabbit eye was shown following an acute immunological inflammatory reaction induced by a single intravitreal injection of sterile crystallized bovine serum albumin (16). This report preceded a study, also in rabbits, demonstrating that an acutely traumatized eye shows an irritative response characterized by hyperaemia of the conjunctiva and iris, miosis and disruption of the blood-aqueous barrier. One of the signs of blood-aqueous barrier disruption is an increased concentration of blood proteins in the aqueous humour. Using a relevant pharmacological model a significant reduction in protein concentration in the aqueous humour could be demonstrated by pretreating the animal with a rectal

dose of acetosal (acetylsalicylic acid; 600mg) (17). Stabilization of the blood-aqueous barrier in the human eye with acetosal administered orally (4 doses of 650mg; 3 before and one on completion of ocular surgery) was reported in 1975 (18).

Levels of prostaglandin-like activity in aqueous humour samples correlated well with the clinical intensity of uveitis. This in contrast to patients with cataract whose aqueous humour was essentially devoid of activity when the eyes were uninflamed, and low in activity when treated with corticosteroids (19).

In vitro inhibition of rabbit prostaglandin synthase systems of various organs, including the eye, by indomethacin was reported in 1974 (20). Tissue homogenates of the iris and the ciliary body (anterior uvea), the conjunctiva, the cornea and retina were prepared; spleen and kidney (medulla) were also investigated. The inhibitory effect of indomethacin was clearly demonstrated and the compound showed differential inhibitory capacity. The retinal enzymes were least susceptible to inhibition followed by iris and ciliary body (twofold more) and the conjunctiva (six fold more). This also raised the possibility that prostaglandins are involved both in external as well as internal ocular inflammation.

The potential complication reported by our ophthalmologists that could arise after cataract surgery, cystoid macular edema, seemed linked to the release of prostaglandins. Thus, in the event of adequate permeation of indomethacin through the cornea, the edema should be prevented by topical administration of eyedrops. In 1972 it has been demonstrated by application of 100 microgram radiolabelled indomethacin to the cornea (either in aqueous suspension form or in oily solution) that the drug could be detected in the cornea, aquous humor, iris, choroids and retina of the rabbit eye (21). An inflamed eye gave rise to enhanced penetration. In 1983 it was subsequently shown, by use of topically applied radiolabelled indomethacin (2% suspension in sesame oil, including 17% ethanol) on phakic and aphakic rabbit eyes, that penetration into the vitreous took place; the concentration in the vitreous was higher for the aphakic eye. Concentrations in retina and choroid were the same for both conditions, suggesting a pathway other than diffusion through the vitreous to reach these tissues. Aqueous humour concentrations were sufficient to inhibit prostaglandin synthesis in either situation (22).

Indomethacin, [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid, molecular weight 357.8 dalton, pKa 4.5, is practically insoluble in water. In aqueous buffers at pH 7.5 - 8.0 it can be rendered soluble (23). In basic solutions hydrolysis of indomethacin occurs into 5-methoxy-2-methylindolyl-3-acetic acid and 4-chlorobenzoic acid (24,25,26). These substances are pharmacologically inactive. In the European pharmacopea (1997) 4-chloro-benzoic acid is mentioned as an impurity.

Pharmacokinetics of indomethacin are as follows. The major route of elimination is by transformation in the liver and involves glucuronidation, O-demethylation and N-deacylation. The major (inactive) metabolites are desmethyl indomethacin, deschlorobenzoyl indomethacin and their glucuronides. Protein binding is more than 90%. Volume of distribution is 0.12 L/kg; clearance is 1-2 mL/min/kg with a half-life of 6 hours. The compound is excreted unchanged in urine for 30%.

Indomethacin was introduced into the field of ophthalmology in different types of formulations including a solution in sesame oil and an ophthalmic aqueous suspension (1,27). Concentrations in suspension eyedrops varied between 0.5% to 1% and in oily solutions from 0.1 to 1%. In the Dutch literature several formulations of indomethacin eyedrops followed the first international reports (vide supra) on the prevention of cystoid macular edema after lens extraction (28,29,30). As use expanded in the clinic, reports indicated that the prepared solutions, being a suspension or an aqueous solution, were irritating to the eye (burning sensation). A reduction in concentration was suggested from 1% to 0.2% or 0.1% to prevent this undesirable effect. In 1981 it was shown that four different indomethacin suspension eyedrops, all being 0.5% in concentration, differed in prostaglandin synthase inhibiting activity, which was attributed to the differences in physicochemical properties. It was concluded that the use of eyedrops as a suspension yields irreproducible results from the pharmacokinetic point of view and gives rise to subjective complaints of irritation in the eye (31).

In 1984 Indoptol®, an aqueous eyedrop suspension of 1% indomethacin, was introduced to the Dutch market and in 1986 Indocid® of comparable composition was introduced in France. In 1987 a second presentation of indomethacin followed in France in the form of Indocollyre® (0,1%), which was introduced in The Netherlands in 1994. This formulation contains indomethacin as a lyophilized (freeze-dried) product which is brought into solution by addition of a sterile borate buffer. In the international literature aqueous formulations of indomethacin eyedrops have been published (32,33,34) reflecting the need for a more suitable and reliable pharmaceutical preparation. Ongoing own research with different bases, L-Lysine, D-Lysine, L-Arginine, D-Arginine, and Tromethamol (not published), to provide an indomethacin solution with an acceptable shelflife, did not provide suitable pharmaceutical alternatives. They all were aqueous solutions in order to circumvent the irritating properties of the suspension based eyedrops and the sesame oil based solution causing blurring of vision by difference in refractive index. However, our originally introduced solution (29) without extra pharmaceutical excipients and having a concentration of indomethacin of 0.1% remained the mainstay of the eye clinic. This solution was tested in a pharmacological setting in the rabbit eye using a paracentesis model of removing the aqueous humor and measuring the influx of protein and fluorescein into the secondary aqueous humor (35). The results

showed, in a concentration of indomethacin as low as 0.05%, 90 - 100% pharmacological efficacy in inhibiting fluorescein and protein influx (36). The indomethacin 0.1% formulation was incorporated in the Dutch national formulary (FNA) in 1986. Inpracticalities with indomethacin in aqueous solution - no sterilization possible and a short shelflife - prompted us to investigate the possibility in formulating eyedrops based on a different NSAID. In 1990 topically applied S(+) ibuprofen was reported to be effective in a rabbit model of interleukin-1 (37) or paracentesis induced uveitis (38) at relatively elevated concentrations (0.9% and 0.8% respectively). Also with S(+) naproxen, marketed by Syntex as enantiomeric pure NSAID, an anti-inflammatory effect of eyedrops (0,5%) was demonstrated experimentally (39).

In our quest for a pharmaceutically more acceptable solution of an NSAID, we turned to the USP in which a flurbiprofen ophthalmic solution is mentioned.

Ophthalmic solutions of flurbiprofen, diclofenac, and indomethacin (pH 7.5), have been subjected to research in rabbit eyes to investigate the maximal effect in preventing breakdown of the blood-aqueous barrier (40). Effective doses [nmol] per eye resulting in 50% inhibition (ID_{50}) of influx of protein and of fluorescein into secondary aqueous humor after paracentesis corresponded well for indomethacin and flurbiprofen (12 nmol for flurbiprofen, 11 nmol for indomethacin, and 8.0 nmol for flurbiprofen and 9.0 nmol for indomethacin, respectively).

In a comparative test of 11 nonsteroidal anti-inflammatory compounds in 0.01% solution, using the rabbit paracentesis model, flurbiprofen proved to be the most effective, showing a half-life of the inhibitory effect of 10 hours (41). A speciality, Ocufen®, containing 0.03% flurbiprofen sodium $2H_2O$ (equivalent to 0.024% flurbiprofen acid), is on the market in the United States since 1987 for inhibition of intraoperative miosis (42). Ocuflur® of comparable composition, marketed in Belgium, is also indicated for use in intraoperative inhibition of miosis, treatment of inflammation as a result of surgical intervention or trabeculoplasty by laser treatment and for prevention of cystoid macular edema after cataract surgery.

We embarked on a study to manufacture flurbiprofen eyedrops by protocol of june 1992. A letter of consent to aid the project (9206SO.008) was issued January 8th 1993 by the SWOR (Stichting ter bevordering van Wetenschappelijk Onderzoek in ziekenhuis Rijnstate).

REFERENCES

1. Miyake K. Prevention of cystoid macular edema after lens extraction by topical indomethacin (I). Albrecht v. Graefes Arch Klin Exp Ophthal 1977;203:81-8.
2. Mochizuki M, Sawa M, Masuda K. Topical indomethacin in intracapsular extraction of senile cataract. Jpn J Ophthalmol 1977;21:215-26.
3. Miyake K. Prevention of cystoid macular edema after lens extraction by topical indomethacin (II): a control study in bilateral extractions. Jpn J Ophthalmol 1978;22:80-94.

4. Miyake K, Sugiyama S, Norimatsu I, Ozawa T. Prevention of cystoid macular edema after lens extraction by topical indomethacin (III): Radioimmunoassay measurement of prostaglandins in the aqueous during and after lens extraction procedures. Albrecht v. Graefes Arch Klin Exp Ophthal 1978;209:83-8.
5. Sholiton, DB, Reinhart WJ, Frank KE. Indomethacin as a means of preventing cystoid macular edema following intracapsular cataract extraction. Am Intra-ocular Implant Soc J 1979;5:137-40.
6. Irvine SR. A newly defined vitreous syndrome following cataract surgery. Interpreted according to recent concepts of the structure of the vitreous. Am J Opthalmol 1953;36:599-619.
7. Selye H. Syndrome produced by diverse nocuous agents. Nature 1936;138:32.
8. Chandler JW, Gillette TE. Immunologic defense mechanisms of the ocular surface. Ophthalmology 1983;90:585-91.
9. Neufeld AH and Sears ML. Prostaglandin and eye. Prostaglandins 1973;4:157-75.
10. Ambache N. Properties of irin, a physiological constituent of the rabbit's iris. J Physiol 1957;135:114-32.
11. Ambache N. Further studies on the preparation, purification and nature of irin. J Physiol 1959;146:255-94.
12. van Dorp DA, Jouvenaz GH, Struijk CB. The biosynthesis of prostaglandin in pig eye iris. Biochim Biophys Acta 1967;137:396-9.
13. Posner J. The release of prostaglandin E_2 from the bovine iris. Br J Pharmacol 1968;34:163P-4P.
14. Waitzman MB. Possible new concepts relating prostaglandins to various ocular functions. Survey of Ophthalmology 1970;14:301-26.
15. Vane JR. Inhibition of prostaglandinsynthesis as a mechanism of action for aspirin-like drugs. Nature 1971;231:232-5.
16. Eakins KE, Whitelocke RAF, Perkins ES, Bennett A, Unger WG. Release of prostaglandins in ocular inflammation in the rabbit. Nature New Biology 1972;239:248-9.
17. Neufeld AH, Jampol LM, Sears ML. Aspirin prevents the disruption of the blood-aqueous barrier in the rabbit eye. Nature 1972;238:168-9.
18. Zimmerman TJ, Gravenstein N, Sugar A, Kaufman HE. Aspirin stabilization of the blood-aqueous barrier in the human eye. Am J Ophthalmol 1975;79:817-9.
19. Eakins KE, Whitelocke RAF, Bennett A, Martenet AC. Prostaglandin-like activity in ocular inflammation. BMJ 1972;3:452-3.
20. Bhattacherjee P, Eakins KE. Inhibition of the prostaglandin synthase systems in ocular tissues by indomethacin. Br J Pharmac 1974;50:227-30.
21. Hanna C, Sharp JD. Ocular absorption of indomethacin by the rabbit. Arch Ophthal 1972;88:196-8.
22. Green K, Bowman K, Luxenberg MN, Friberg TR. Penetration of topical indomethacin into phakic and aphakic rabbit eyes. Arch Ophthalmol 1983;101:284-8.
23. Katz IM. Indomethacin. Ophthalmology 1981;88:455-8.
24. Krasowska H, Krowczynski L, Bogdanik Z. The assay of indomethacin in the presence of its hydrolytic degradation products. Pol J Pharmacol Pharm 1973;1973;25:417-21.
25. Kahns, AH, Jensen, PB, Mørk N, Bundgaard H. Kinetics of hydrolysis of indomethacin and indomethacin ester prodrugs in aqueous solution. Acta Pharm Nord 1989;1:327-36.
26. Tomida H, Kuwada N, Tsuruta Y, Kohashi K, Kiryu S. Nucleophilic aminoalcohol-catalyzed degradation of indomethacin in aqueous solution. Pharm Acta Helv 1989;64:312-5.
27. Yanuzzi LA, Landau AN, Turtz AI. Incidence of aphakic cystoid macular edema with the use of topical indomethacin. Ophthalmology 1981;88:947-54.
28. Lute NP, Vyth A, De Keizer RJW. Indometacine oogdruppels 0,5%. Pharm Weekbl 1980; 115:1663-4.
29. Van Nispen tot Pannerden EBLM, Van Sorge AA. Waterige oogdruppels met indometacine in oplossing; "corpora agunt nisi soluta". Pharm Weekbl 1981;116:386-7.

30. Cox HLM van der Graaf H. Indometacine-oogdruppels als oplossing. Pharm Weekbl 1981;116:387-8.
31. Oosterhuis JA, van Haeringen NJ, Glasius E, van Delft JL, Swart-van den Berg M. The effect of indomethacin on the anterior segment of the eye after paracentesis. Documenta Ophthalmologica 1981;50:303-13.
32. Bechetoille A, Chabanais JL, Jallet G, Saraux H. Contusion et perméabilité de la barrière héma-to-aqueuse à la fluorescéine. Influence d' un pré-traitement par l'indométacine locale. J Fr Ophthalmol 1978;1:139-43.
33. Liou S-W, Yen R-J. The effect of 0.1% indomethacin eyedrops on cataract surgery. J Ocul Pharmacol 1991;7:77-81.
34. Kahan LI, Bögi J, Farkas A, Tüdos F, Imre Gy. Az Indosol – nagy terápiás hatású nemszteroid gyulladásgátló – ismertetése. Acta Pharmaceutica Hungarica 1994; 64:125-9.
35. Van Haeringen NJ, Oosterhuis JA, van Delft JL, Glasius E and Noach EL. A comparison of the effects of non-steroidal compounds on the disruption of the blood-aqueous barrier. Exp Eye Res 1982;35:271-7.
36. Van Sorge AA, Van Nispen tot Pannerden EBLM, Janssen HWM. Oogdruppels met lage con-centratie indometacine: bereidingsvoorschrift en onderzoek naar de werkzaamheid. Pharm Weekbl 1986;121:1039-46.
37. Tilden ME, Boney RS, Goldenberg MM and Rosenbaum JT. The effects of topical S[+]-ibuprofen on interleukin-1 induced ocular inflammation in a rabbit model. J Ocul Pharmacol 1990;6:131-5.
38. Tjebbes GWA, van Delft JL, Barthen ER, van Haeringen NJ. d-Ibuprofen in ocular inflammation induced by paracentesis of the rabbit eye. Prostaglandins 1990;40:29-33.
39. Stampinato S, Marino A, Bucolo C, Canossa M, Bachetti T, Mangiafico S. Effects of sodium naproxen eyedrops on rabbit ocular inflammation induced by sodium arachidonate. J Ocul Pharmacol 1991,7:125-133.
40. Van Haeringen NJ, Oosterhuis JA, van Delft JL, Glasius E and Noach EL. A comparison of the effects of non-steroidal compounds on the disruption of the blood-aqueous barrier. Exp Eye Res 1982;35:271-7.
41. Van Haeringen NJ, Glasius E, Oosterhuis JA, van Delft JL. Drug prevention of blood-aqueous barrier disruption. Ophthalmic research 1983;15:180-4.
42. Anonymous. Flurbiprofen – an ophthalmic NSAID. The Medical Letter 1987;29:58-9.

SCOPE OF THE THESIS

The studies described in this thesis were performed to investigate and to evaluate (1) the pharmaceutical application of flurbiprofen in eyedrops and (2) the pharmacology of this non-steroidal anti-inflammatory drug - both the racemic form and the individual enantiomers -, with special reference to the constitutive and inducible cyclooxygenases, COX-1 and COX-2, respectively.

Chapters 2 and 3 cover the pharmaceutical aspects of S(+) flurbiprofen eyedrops, i.e. the formulation, the analysis (including the development of an enantiomeric assay), and the chemical and enantiomeric stability under different conditions and periods of time.

In Chapter 4 the specificity of flurbiprofen and its enantiomers for inhibition of PGE_2 production by COX-1 in the bovine iris/ciliary body was investigated including the possibility of chiral inversion during the period of incubation.

The interaction with the COX-1 and COX-2 isozymes in whole human blood, an extra-ocular matrix, was addressed in Chapter 5. COX-1 activity was monitored by measuring TxB_2 (the stable metabolite of TxA_2) production from platelets whereas COX-2 activity was determined using PGE_2 production in monocytes, following induction of this isozyme by LPS. In Chapter 6 the interaction of S(+) flurbiprofen with COX-1 and COX-2 in the human iris was studied. After LPS-treatment for 24h, substantial amounts of COX-2 immunoreactivity could be visualized for the first time in human iris/ciliary body preparations. Remarkably, S(+) flurbiprofen showed a 3,600-fold higher potency for inhibiting COX-1 compared to COX-2. Furthermore, the susceptibility of human iris COX-1 for inhibition by S(+) flurbiprofen was 70-fold higher than of COX-1 in human blood.

In Chapter 7 ^{99m}Tc-labeled diflunisal eyedrops were applied in the human eye in an attempt to visualize the internal structures having high(est) COX-activity. Diflunisal was used for radiolabeling instead of S(+) flurbiprofen because the labeling efficiency of the latter compound was insufficient (Chapter 8). Scintigraphic activity surrounding the pupil indeed provided clear evidence of visualization of the iris/ciliary body.

In the final Chapter the occurrence of alternative splicing of COX-1 in RNA in the human iris was explored, as a possible explanation of the remarkably high affinity of S(+) flurbiprofen for COX-1 reported in Chapter 6.